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Increasing microbial carbon use efficiency with warming predicts soil heterotrophic respiration globally

Running Title: Microbe carbon use predicts soil respiration

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Abstract

The degree to which climate warming will stimulate soil organic carbon (SOC) losses via heterotrophic respiration remains uncertain, in part because different or even opposite microbial physiology and temperature relationships have been proposed in SOC models. We incorporated competing microbial carbon use efficiency (CUE)–mean annual temperature (MAT) and enzyme kinetic–MAT relationships into SOC models, and compared the simulated mass-specific soil heterotrophic respiration rates with multiple published datasets of measured respiration. The measured data included 110 dryland soils globally distributed, and two continental to global-scale cross-biome datasets. Model–data comparisons suggested that a positive CUE–MAT relationship best predicts the measured mass-specific soil heterotrophic respiration rates in soils distributed globally. These results are robust when considering models of increasing complexity and competing mechanisms driving soil heterotrophic respiration–MAT relationships (e.g., carbon substrate availability). Our findings suggest that a warmer climate selects for microbial communities with higher CUE, as opposed to the often hypothesized reductions in CUE by warming based on soil laboratory assays. Our results help to build the impetus for, and confidence in, including microbial mechanisms in soil biogeochemical models used to forecast changes in global soil carbon stocks in response to warming.

1 Introduction

Climate warming is expected to accelerate soil carbon dioxide (CO₂) release to the atmosphere via microbial heterotrophic respiration (Bond-Lamberty, Bailey, Chen, Gough, & Vargas, 2018; Dorrepaal et al., 2009; Giardina, Litton, Crow, & Asner, 2014; Heimann & Reichstein, 2008), representing a positive climate–carbon (C) cycle feedback embedded in most Earth System Models (ESMs) (Ciais et al., 2014; Wieder et al., 2015). However, the confidence in the strength of this projected feedback is low because of uncertainties related to the representation of microbial processes in these models (Bradford et al., 2016). Specifically, soil biogeochemical sub-models in ESMs typically treat microbial physiological responses to temperature implicitly as a first-order process, whereby respired CO₂ is proportional to the amount of soil C stocks (Todd-Brown et al., 2013; Wieder et al., 2015). A wealth of emerging experimental and observational data have led to questions about the validity of this approach, suggesting instead that a diverse suite of microbial responses to the thermal regime drives soil C losses with warming (Frey, Lee, Melillo, & Six, 2013; Shannon B. Hagerty et al., 2014; Karhu et al., 2014; Walker et al., 2018). Furthermore, a proliferation of new models explicitly including microbial physiological and community dynamics show that soil C persistence and vulnerability to warming strongly depend on how such microbial processes are represented (Allison, Wallenstein, & Bradford, 2010; Ballantyne IV & Billings, 2018; Georgiou, Abramoff, Harte, Riley, & Torn, 2017). However the validity of these representations, and hence their ability to build confidence in hypothesized microbial and soil C responses to warming, is still compromised because projections are rarely tested against measured data across a wide range of temperatures. While the performance of soil organic

carbon (SOC) models explicitly addressing microbial community-level properties (e.g., community composition, and density-dependent microbial turnover) has been recently tested against wide ranges of measured data in a couple of studies (Georgiou et al., 2017; Johnston & Sibly, 2018), this is not the case for models incorporating physiological attributes of microbes such as carbon use efficiency (CUE).

Previous attempts to represent microbial physiology have focused on testing SOC models of increasing complexity that couple microbial processes with soil C turnover. To do so, these modeling exercises use relationships between temperature and physiological parameters such as microbial CUE and extracellular enzyme kinetics (Allison et al., 2010; Li, Wang, Allison, Mayes, & Luo, 2014; Walker et al., 2018; Wang, Post, & Mayes, 2013). For example, microbial CUE is an emergent property of multiple microbial processes (Shannon B Hagerty, Allison, & Schimel, 2018; Xu et al., 2014); it partitions the flow of soil C into microbial growth and heterotrophic respiration (Giorgio & Cole, 1998), two processes that are strongly affected by temperature (Kirschbaum, 1995; Romero-Olivares, Allison, & Treseder, 2017). Laboratory soil assays suggest that microbial respiration rates increase more with temperature than the rates of microbial growth, thus CUE has been hypothesized to decrease with warming (Kirschbaum, 1995; Steinweg, Plante, Conant, Paul, & Tanaka, 2008; Tucker, Bell, Pendall, & Ogle, 2013). However, support for such a decreasing CUE driven by warming is either weak or lacking in field studies (Blagodatskaya, Blagodatsky, Khomyakov, Myachina, & Kuzyakov, 2016; Dijkstra et al., 2011; Frey et al., 2013). Furthermore, recent empirical findings from regional to global scales suggest CUE increases with mean annual temperature

(MAT, spatial difference between sites) of the source environments of microbes (Sinsabaugh, Moorhead, Xu, & Litvak, 2017; Sinsabaugh et al., 2016; Takriti et al., 2018). MAT represents the ambient thermal regime of microbes, and it is more relevant than assay temperature for long-term global C models such as those typically used to forecast the response of soil C stocks to warming (e.g., Xu et al., 2017). As most microbes live below their maximum physiological thermal limitation, they may shift towards more rapidly growing organisms, and thus more efficiently growing communities, under a higher MAT regime (Bradford, 2013; Ng, 1969; Pirt, 1965). A positive CUE-MAT relationship may also be indirectly driven by resource availability as plant C inputs are typically higher in warmer climates (Sinsabaugh et al., 2017; Sinsabaugh et al., 2016; Takriti et al., 2018). To help tease out which of these mechanisms is likely to be operating under natural conditions, SOC models should test such competing hypotheses by evaluating their ability to predict patterns observed with empirical data at the regional to global scale.

Here we incorporated relationships between physiological attributes of microbial communities and MAT (Figure 1a) into both first-order and microbial-explicit SOC models. We then tested the ability of these models to predict mass-specific soil heterotrophic respiration (R_{mass} , respiration rate per unit of microbial biomass C) of 110 dryland soils measured in a short term laboratory incubation under optimal moisture (Dacal, Bradford, Plaza, Maestre, & García-Palacios, 2019). This global dryland dataset is highly suited to test competing expectations as to how microbial physiology should respond to MAT. This is because the dataset captures broad ranges in MAT across sites (ranging from -1.8°C to 28°C ,

for all continents except Antarctica) and fine-scale differences in C substrate availability and microclimate within a given site (e.g. SOC within a site may be up to ~45% higher under vegetation compared to adjacent bare ground areas; Ochoa-Hueso et al., 2018). Further, R_{mass} was estimated using an experimental approach that controls for the main factors driving the microbial heterotrophic respiration-temperature relationship (i.e. microbial biomass and substrate availability; Dacal et al., 2019). We also compared our model simulated R_{mass} to continental-scale R_{mass} measurements for soils collected across boreal to tropical climates (Bradford et al., 2019) and a global-scale R_{mass} dataset compiled from published soil incubation studies (Xu et al., 2017).

We focused on two sets of hypotheses testing whether relationships between CUE and MAT (Allison et al., 2010; Sinsabaugh et al., 2016), and between microbial extracellular enzyme kinetics and MAT (Allison, Romero-Olivares, Lu, Taylor, & Treseder, 2018; Allison et al., 2010; German, Marcelo, Stone, & Allison, 2012; Hochachka & Somero, 2002), are positive or negative (Figure 1). Additionally, we tested the robustness of these hypotheses against expected controls on respiratory patterns in SOC models (Crowther et al., 2016; Walker et al., 2018), such as microbial complexity (i.e. first order vs. microbial models) and C substrate availability (i.e. vegetated patches vs. open areas, and excess vs. ambient substrate supply).

2 METHODS

2.1 Hypothesized microbial physiology – temperature relationships

In the first set of hypotheses (H1), we tested positive vs. negative CUE–MAT relationships without modifying the maximal activity (V_{\max}) and the half-saturation constant (K_m) of soil microbial extracellular enzyme kinetics (Figure 1b). We defined MAT as the long-term ambient temperature regime of a site; it is commonly calculated as the mean of the annual temperatures for a 30-year period (Fick & Hijmans, 2017).

H1a: CUE increases with warming (Sinsabaugh et al., 2017; Sinsabaugh et al., 2016; Takriti et al., 2018) following this equation:

$$\text{CUE} = \exp(-1.58 + 0.015 \times \text{MAT}) \quad (1)$$

H1b: CUE decreases with warming based on laboratory soil incubation (Allison et al., 2010; Li et al., 2014; Steinweg et al., 2008). Therefore, we also assumed that CUE decreases with the ambient thermal regime of microbes' habitats following this equation:

$$\text{CUE} = 0.63 - 0.016 \times \text{MAT} \quad (2)$$

The maximal activity and substrate affinity of microbial extracellular enzymes have also been used to explain patterns in soil heterotrophic respiration across climatic gradients (Allison et al., 2018; German et al., 2012; Hochachka & Somero, 2002). In the second set of hypotheses (H2, Figure 1b), we tested positive vs. negative extracellular enzyme kinetics (V_{\max} and K_m) –MAT relationships while fixing CUE at the 20°C reference MAT value, i.e.

CUE = $\exp(-1.58 + 0.015 \times 20)$. Currently, SOC models do not include a mechanism for the influence of soil stoichiometry (e.g. nitrogen to phosphorus ratio) on extracellular enzyme kinetics (Allison et al., 2010; Georgiou et al., 2017; Li et al., 2014). Therefore, we assumed that all resource stoichiometry and metabolic constraints remained constant across the MAT gradient, and all enzymes responded in parallel.

H2a: When measured at the same laboratory assay T, V_{\max} and K_m may be lower for enzymes from warmer habitats (German et al., 2012; Hochachka & Somero, 2002). We assumed K_m decreases with warming at a rate of 1% per °C following this equation:

$$K_m \times (1 - 0.01 \times \text{MAT}) \quad (3)$$

Sinsabaugh et al. (2014) suggested that V_{\max} and K_m generally change in the same direction, and K_m could show a greater change than V_{\max} . We assumed that V_{\max} decreases with MAT at a lower rate of 0.5% following this equation:

$$V_{\max} \times (1 - 0.005 \times \text{MAT}) \quad (4)$$

H2b: V_{\max} and K_m usually increase with laboratory assay temperature (Allison et al., 2018; German et al., 2012; Hochachka & Somero, 2002); we also assumed that both K_m and V_{\max} increase with MAT following this equation:

$$K_m \times (1 + 0.01 \times \text{MAT}), \text{ and } V_{\max} \times (1 + 0.005 \times \text{MAT}) \quad (5)$$

Equations and parameters for calculating K_m and V_{\max} are reported in the Supplementary Information (Method S1 and Table S1).

2.2 Soil organic carbon models

We used four soil organic carbon (SOC) models that vary in structure and complexity (Figure S1), including a conventional first-order decomposition model (Parton, Stewart, & Cole, 1988) and three microbial-explicit models (Allison et al., 2010; Georgiou et al., 2017; Li et al., 2014; Wang et al., 2013). Equations and parameters of the four SOC models used are given in the Supplementary Information (Method S1 and Table S1). All the SOC models used run at an hourly time step. The first-order model separates the soil C into three pools, including SOC, dissolved organic C (DOC) and microbial biomass C (MBC) (Parton et al., 1988). This model describes the transfers of C between these soil C pools and to the atmosphere (i.e. respired CO₂) using first-order linear decay rates that are modified by environmental scalars. The first-order model does not include an explicit CUE, but the transfer coefficients that specify partitioning of fluxes into CO₂ versus soil C pools are analogous (Allison et al., 2010; Li et al., 2014). The CO₂ respiration rate is the sum of the proportion of C fluxes that do not enter soil C pools (Ciais et al., 2014; Parton et al., 1988). In the microbial-explicit models, the rates of SOC decomposition and CO₂ respiration depend on the sizes of both the soil C and the decomposer microbe (MBC) pools. The microbial-explicit models include a CUE that portions soil C into microbial growth and heterotrophic respiration (Georgiou et al., 2017; Li et al., 2014; Wang et al., 2013). The microbial models also explicitly include an enzyme pool (Georgiou et al., 2017; Li et al., 2014; Schimel & Weintraub, 2003; Wang et al., 2013).

2.3 Soil heterotrophic respiration measurements

Dacal et al. (2019) tested the relationship between soil heterotrophic respiration and MAT by incubating soils from 110 globally-distributed dryland sites in the laboratory. Briefly, soils were sampled separately under the canopy of vegetation and in bare ground areas devoid of perennial plants. Soils were incubated under controlled laboratory conditions with or without C substrate supply, where glucose (at a dose of 10 mg C g^{-1} dry soil) and sterile deionized water were added, respectively. Soils were incubated at three assay temperatures (10°C , 20°C and 30°C). The respiration rates were measured during the last 5 h of a 10 h-incubation period using the MicroRespTM technique (Campbell, Chapman, Cameron, Davidson, & Potts, 2003). The short incubation time used prevents the acclimation of soil heterotrophic respiration to the assay temperatures of laboratory conditions (Dacal et al., 2019), an approach similar to those used in plants, cultured microbes and other soil studies (Atkin & Tjoelker, 2003; Bradford, Watts, & Davies, 2010; Hochachka & Somero, 2002; Tucker et al., 2013). Dacal et al. (2019) built a linear mixed-effect regression model to test for the effects of MAT, assay temperatures, and micro-environment on potential respiration rates in the 110 drylands, where microbial biomass was statistically controlled (i.e. included as a covariate in the model). They estimated the effect sizes of MAT, assay temperatures and micro-environment using coefficients of the regression. We used the effects of MAT, assay temperatures and micro-environment on R_{mass} from Dacal et al. (2019) to confront our SOC model simulations (Figure 1).

We also compared our SOC model simulations to other two large-scale studies (Bradford et al., 2019; Xu et al., 2017). Bradford et al. (2019) measured respiration rates for soils from 11 sites in the laboratory with standard temperatures, moisture and excess C substrate. The sites used in this study spanned from boreal to tropical climates, with a mean annual temperature varying from -2.0°C to 21.7°C . Bradford et al. (2019) also built a linear mixed-effect regression model to test for the effects of MAT, assay temperatures, and microbial biomass on soil respiration rates. Xu et al. (2017) compiled a global R_{mass} dataset from published soil incubation studies. Due to the different incubation temperatures used among publications, Xu et al. (2017) adjusted R_{mass} rates measured at different incubation temperatures to their long-term average site soil temperatures using a temperature sensitivity $Q_{10} = 2$. They found that the adjusted R_{mass} rates increase exponentially with the long-term average site soil temperatures, $\log(R_{\text{mass}}) = -3.1102 + 0.0214 \times T_{\text{soil}}$ (where T_{soil} is the site soil temperature; $R^2 = 0.0698$; $P < 0.001$).

2.4 Model simulations and comparisons with empirical data

We incorporated the four microbial physiology vs. MAT relationships described in the *Hypothesis testing* section above into SOC models, and tested their ability to simulate soil microbial heterotrophic respiration measured in soils from 110 global drylands (Figure 1). To increase the consistency between the model outputs and the short-term soil incubations used to test them, we (i) established 31 “theoretical” sites across the MAT gradient present in the dryland sites surveyed (ranging from -2°C to 28°C , one site for each 1°C difference in MAT), (ii) ran SOC models for 10 h for each of the 31 theoretical sites and four hypothesized

relationships at 10°C, 20°C and 30°C assay temperatures, and (iii) set the C inputs to zero during the 10 h simulations. As soil microbial biomass may not change during this short timescale, we also ran the models for one year to explore the longer-term changes in soil heterotrophic respiration, microbial biomass and organic C. The conventional first-order model represents the decomposition rate of each pool as a first-order decay process, where the decay increases exponentially with temperature according to the Arrhenius relationship (see Method S1 Equation 1 for the calculation). In the microbial-explicit models, soil microbial extracellular enzyme kinetics (V_{\max} and K_m) also have an Arrhenius dependence on temperature. We ran the SOC models with temperature = 10°C, 20°C and 30°C, respectively, which is analogous to the three assay temperatures used in the soil incubations studies.

To mimic Xu et al.'s (2017) analyses, which adjusted R_{mass} rates at different incubation temperatures to the long-term average temperatures of the soils studied, we simulated R_{mass} by setting an assay temperature (T) that matched the site's long-term MAT value. We compared our simulations to those reported in Xu et al. (2017), assuming that the long-term site soil temperature equals its MAT. Bradford et al. (2019) and Dacal et al. (2019) also conducted similar analyses. They developed a linear mixed-effect model between respiration rates and MAT, assay temperature, microbial biomass. They then estimated R_{mass} for each soil assuming an assay temperature that matched the MAT value of its source site.

We accounted for the influence of changes in microbial biomass and in C substrate availability, which are major mechanisms underlying temperature effects on soil heterotrophic respiration (Bradford et al., 2008; Erhagen, Ilstedt, & Nilsson, 2015; Walker et al., 2018). To do so, we calculated the R_{mass} rates as the respiration rates per unit microbial biomass C ($\text{g C g microbial biomass}^{-1} \text{ day}^{-1}$). This is necessary to control for microbial biomass changes, as biomass itself is a factor that regulates soil heterotrophic respiration (Bradford et al., 2008; Walker et al., 2018). To account for changes in C substrate availability, we used two sets of initial carbon pool sizes; i.e. equilibrium steady state vs. enlarged C pool sizes. First, we used each model's equilibrium steady state C pool sizes at a 20°C reference temperature and $\text{CUE} = \exp(-1.58 + 0.015 \times 20)$ as its initial values (see Table S2 for the values used). Second, we enlarged the equilibrium steady state DOC pool size by 100 times to remove substrate limitation, representing a scenario of C substrate in excess of microbial demand. We compared the two sets of model simulated R_{mass} rates to those measured in the soil laboratory incubations with water and excess glucose ($10 \text{ mg C g}^{-1} \text{ dry soil}$) as substrates (Dacal et al., 2019), resembling ambient and excess C substrates scenarios, respectively.

We derived the steady-state pool sizes from the analytical solutions for the simpler SOC models (the first-order model, and the two- and four-pool microbial models, see Method S1), and from a spin-up model run for the more complex six-pool microbial model (Allison et al., 2010; Georgiou et al., 2017; Li et al., 2014; Ye, Reynolds, & Li, 2014). To facilitate comparisons among the hypotheses and models used, we reported the relative changes (%) in simulated R_{mass} as compared to equilibrium values at a 20°C reference temperature of the

respective models, as done in prior studies (Georgiou et al., 2017; Li et al., 2014). As the three microbial-explicit models displayed essentially similar patterns (Figure S2), we only show in the main text the results of the model that includes four C pools. The complexity of the four-pool microbial model is comparable to that of the first-order model; the two models share three similar C pools, i.e. SOC, DOC and MBC (Figure S1a and S1c), which allows a more direct inter-model comparison.

Finally, we also explored potential mechanisms behind a positive CUE – MAT relationship using the measured R_{mass} rates in the vegetated patches and open areas. Vegetated patches in drylands have lower temperature microclimates than bare ground areas due to shading and evaporative cooling (Maestre, Bautista, Cortina, & Bellot, 2001; Molles, Cahill, & Laursen, 2015), and higher resources because plant-C inputs and SOC are concentrated there (Dacal et al., 2019; Molles et al., 2015; Ochoa-Hueso et al., 2018). A positive CUE – MAT relationship may arise because higher temperatures and/or greater resource availability select for more growth-efficient microbial communities in warmer climates (Bradford, 2013; Ng, 1969; Sinsabaugh et al., 2017; Sinsabaugh et al., 2016). We simulated these mechanisms as (i) CUE in the vegetated patches = $\exp[-1.58 + 0.015 \times (\text{MAT} - 10)]$, assuming that a vegetated patch is 10°C cooler than its corresponding open area (Molles et al., 2015), and (ii) CUE in the vegetated patches = $\exp(-1.58 + 0.015 \times \text{MAT}) \times 1.45$, assuming that SOC is ~45% higher under vegetated patches than in open areas (Dacal et al., 2019; Molles et al., 2015; Ochoa-Hueso et al., 2018).

3 Results

Our short-term (10 h) SOC model simulations incorporating a positive CUE–MAT relationship (Hypothesis 1a, Figure 1) are consistent with the R_{mass} patterns observed across 110 global drylands (Figure 2a-c). At a common assay temperature, both the simulated and measured R_{mass} are lower at sites with higher MAT (0.3–1.5% lower per extra °C, $P < 0.05$). Such a R_{mass} pattern is consistent in both the first-order and microbial-explicit SOC models (Figure 2b-c), and is maintained when C substrate is supplied in excess of microbial demand (Figure 2) or at ambient values (Figure S3). The same results were found when the models were simulated for one year (Figure 3a-b). Our model simulations using a negative CUE–MAT relationship (Hypothesis 1b, Figure 1), however, show the opposite R_{mass} –MAT relationship to that observed in the global dryland empirical dataset (Figure 2d-e). That is, R_{mass} increases under this condition with the MAT of the source environment, and this increase is found regardless of the three temperatures at which soils were assayed in the laboratory (Figure 2d-e).

In the longer term (one year), both the first-order and microbial models showed that increasing CUE with MAT also leads to higher microbial biomass carbon (MBC, Figure 3c-d). However, the two models differed in the responses of MBC to assay temperature, and those of SOC to MAT. Microbial biomass C decreased with assay temperature in the first-order model (Figure 3c), while increased with assay temperature (and with smaller effect sizes) in the microbial model (Figure 3d). Soil organic C increased with increasing MAT in the first-order model (Figure 3e), while decreased with MAT in the microbial model (Figure

3f). However, in both models, SOC decreased with increasing assay temperature (Figure 3e-f).

We further tested whether a positive relationship between microbial CUE and MAT arises because of higher temperatures or greater resource availability in warmer climates. When simulating the effects of temperature (i.e. a higher CUE in open areas), we found that R_{mass} was ~5% higher under vegetated patches than in open areas, which is consistent with the patterns observed in global drylands (Figure 4a-c). Conversely, when simulating the resource availability driver (i.e. a higher CUE under vegetated patches), the simulated pattern was the opposite of that observed in global drylands (Figure 4d-e). These results were also consistent regardless of the ambient C substrate scenario considered (Figure S4).

We also tested the ability of soil extracellular enzyme responses to predict the patterns in R_{mass} observed across drylands. When testing a negative V_{max} - and K_m -MAT relationship (Hypothesis 2a, Figure 1b) under C substrate in excess of microbial demand, the simulated R_{mass} at a common assay temperature was lower at higher MAT (0.2–0.5% lower per extra °C, $P < 0.001$; Figure 5a). Such a pattern is consistent with that observed for global drylands (Figure 2a), which the positive V_{max} - and K_m -MAT relationship (Hypothesis 2b, Figure 1b) did not successfully simulate (Figure 5b). However, when considering both scenarios of C substrate in excess and at ambient levels, neither of the two hypothesized relationships predicted a R_{mass} pattern consistent with that found in global drylands (Figure 5 and Figure S5).

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Finally, we simulated the response of R_{mass} to assay temperature. Both models represented the measured increase in R_{mass} with assay temperature, when C substrate was in excess of microbial demand or at ambient levels (Figures 2 and 5, and Figures S3 and S5). The measured R_{mass} was ~40% higher at 20°C than at 10°C assay temperatures, and ~65% higher at 30°C than at 20°C in the 110 global dryland soils (Figure 2a), representing an exponential increase. The two values were comparable for the microbial-explicit model (~50% and ~75%, respectively; Figure 2c), while were larger for the first-order model (~50% and ~90%, respectively; Figure 2b). Such exponential increases were much clearer when we set an assay temperature that matched the site's long-term MAT value in the SOC models (Figure 6). Our comparison to several large-scale empirical datasets further showed that a positive CUE-MAT predicted R_{mass} better than a negative relationship, in both the first-order and microbial-explicit models (Figure 6).

4 Discussion

4.1 Increasing microbial carbon use efficiency with warming predicts soil heterotrophic respiration globally

Combining diverse SOC models (Allison et al., 2010; Li et al., 2014) and multiple published datasets of measured soil respiration rates (Bradford et al., 2019; Dacal et al., 2019; Xu et al., 2017), our study provides a unique test of different assumptions about soil microbial physiology as a function of MAT. We found that a positive CUE–MAT relationship predicted the decreasing mass-specific soil heterotrophic respiration rates with ambient thermal regimes observed in global drylands (Dacal et al., 2019). Such a decreasing pattern of

R_{mass} with MAT has also been found across boreal, temperate and tropical climates (Bradford et al., 2019). Given that there are global analyses suggesting a positive CUE–MAT relationship for soil microbial biomass (Sinsabaugh et al., 2017; Sinsabaugh et al., 2016; Takriti et al., 2018), the CUE–MAT relationship provides a plausible explanation for the observed decreasing R_{mass} with MAT. Our findings suggest that a warmer climate may select for microbial communities with higher CUE, as opposed to the often hypothesized reductions in CUE with warming found in laboratory soil studies (Steinweg et al., 2008; Tucker et al., 2013).

Microbial CUE has been assumed to increase with MAT because resource availability from plant C inputs is typically higher in warmer climates (Sinsabaugh et al., 2017; Sinsabaugh et al., 2016). Our model–data comparison of vegetated patches vs. open areas suggested that the effect of MAT on CUE is robust when considering different levels of C substrate availability. First, the observed pattern that R_{mass} is higher under vegetated patches than in open areas was maintained when C substrate availability is in excess of microbial demand (Figure 4a), as well as when any C substrate limitation was not alleviated (Figure S3a; Bradford et al., 2019; Dacal et al., 2019). Second, vegetated patches have lower MAT, and thus lower CUE, than open areas. Representing this mechanism in our SOC models led to simulated patterns of R_{mass} consistent with those observed in the 110 drylands studied (Figure 4a, d, e). Third, resource availability such as SOC actually decreases, not increases, with MAT across the globe (Delgado-Baquerizo et al., 2013; Xu et al., 2017; Ye, Delgado-Baquerizo, Soliveres, & Maestre, 2019). Therefore, our results are robust when

considering competing mechanisms driving soil respiration–MAT relationships, such as direct vs. indirect C substrate availability effects (Crowther et al., 2016; Walker et al., 2018). However, as the SOC models did not include other resources, such as nitrogen and phosphorus (Xu et al., 2017), we could not conclude definitively that the effect of MAT on CUE is independent of resource availability. Furthermore, soil heterotrophic respiration rates were measured and simulated under optimal moisture, whereas in drylands moisture limitation is a strong control on respiration rates but was not considered in our study. Future work should examine the influence of moisture, especially given that temperature increases in drylands tend to parallel decreases in moisture availability.

Although recognizing CUE is an emergent property of multiple microbial processes (Shannon B Hagerty et al., 2018; Xu et al., 2014), we represented it as a single variable given that the main objective of our study was to confront different assumptions about the response of microbial CUE to warming with empirical data (Figure 1). A single CUE matched the measurement of the empirical soil respiration data used, which were also the sum of different respiratory components (Bradford et al., 2019; Dacal et al., 2019; Xu et al., 2017). Therefore, neither the empirical data nor our modeling approach allows us to distinguish which respiratory component(s) adjusts to MAT, and hence to explain why there should be a positive effect of MAT on CUE. Nevertheless, our results are consistent with theory and data suggesting that microbial life history strategies shift toward more rapidly growing organisms in warmer climates, with maintenance costs remaining relatively constant (Bradford, 2013; Ng, 1969; Pirt, 1965).

4.2 Implications of the model inter-comparisons

Although the first-order and microbial models could represent the observed R_{mass} -MAT pattern, the positive CUE-MAT relationship had different implications for SOC stocks. Microbial biomass does not feedback to SOC decomposition in the first-order model (Ciais et al., 2014; Parton et al., 1988). Thus, SOC increases with MAT in the first-order model (Figure 3) simply because less carbon is respired as a result of an increase in CUE. In the microbial models, an increase in CUE leads to higher microbial biomass, which then produces more enzymes and consumes more SOC (Figure 3). The microbial models then allow a feedback from the microbial pool size to SOC decomposition rates (Georgiou et al., 2017; Li et al., 2014; Schimel & Weintraub, 2003; Wang et al., 2013). The simulated inverse relationship between MAT and SOC stock in the microbial model (Figure 3f) is consistent with that found in soils globally (Xu et al., 2017; Ye et al., 2019). In this regard, the microbial-explicit model is a more accurate representation than the first-order model. Although the soil C stock is not only determined by soil microbial biomass and respiration, but also by factors such as C inputs from vegetation (Crowther et al., 2016; Xu et al., 2014), improving our ability to simulate microbial physiology (i.e. in this case R_{mass}) is necessary if we are to increase confidence in model projections of how temperature changes will affect soil carbon stocks.

In short-term assays, we would expect total respiration and R_{mass} to increase with laboratory assay temperature (Bradford et al., 2019; Dacal et al., 2019; Xu et al., 2017), at least within typical biological temperature ranges, based on the expected positive response of

metabolic rates to temperature (Hochachka & Somero, 2002). Indeed, all models represented the observed exponential increase in R_{mass} with assay temperature, and the microbial-explicit model performed relatively better than the first-order model (Figures 2 and 6). The exponential pattern of soil heterotrophic respiration responses to temperature is a typical output of first-order models where SOC decay rate (and thus CO_2 efflux) is calculated by the Arrhenius relationship (Davidson & Janssens, 2006). By contrast, microbial-explicit models typically use the more complex Michaelis–Menten process to calculate CO_2 efflux (Allison et al., 2010; Buchkowski, Bradford, Grandy, Schmitz, & Wieder, 2017; Li et al., 2014; Manzoni & Porporato, 2009). The Michaelis–Menten reaction rate results from simultaneous changes in substrate concentration (to account for the influence of C substrate availability) and in two kinetic parameters (V_{max} and K_{m} , both are calculated by the Arrhenius relationship). Considering that the SOC models used in our study were not specifically developed and parameterized for any of the three empirical studies used for comparison purposes (Bradford et al., 2019; Dacal et al., 2019; Xu et al., 2017), the agreement between the measured R_{mass} and that simulated by the microbial-explicit model is remarkable (Figures 2 and 6). Hence, our analyses support the explicit representation of microbial physiology in SOC models to simulate accurately both the temporal (assay temperature) and spatial (MAT) response of R_{mass} to climate warming.

4.3 Concluding remarks

Our results indicate that the assumption of a positive CUE–MAT relationship can accurately simulate patterns of mass-specific heterotrophic respiration rates found in multiple empirical studies across a 30°C MAT gradient. Our modeling results are robust to variation in other factors thought to influence CUE and R_{mass} , such as carbon substrate availability (Crowther et al., 2016; Walker et al., 2018). Further, the positive CUE–MAT relationship provides a plausible mechanistic explanation for the observed negative effects of MAT on mass-specific heterotrophic respiration, a pattern found not only in drylands but also from arctic to tropical biomes (Bradford et al., 2019; Dacal et al., 2019). Our results provide novel insights into the mechanisms underlying soil respiration responses to MAT regimes and indicate that SOC models should incorporate a positive CUE–MAT relationship. Doing so would undoubtedly improve the confidence of their predictions, and thus our ability to accurately forecast C stocks and dynamics in a warmer world.

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Figure captions

Figure 1. Diagrammatic overview of our study (a) and of the different hypotheses tested regarding microbial physiology–mean annual temperature (MAT) relationships (b). CUE is the soil microbial carbon use efficiency. V_{\max} and K_m are the maximal activity and the half-saturation constant of soil microbial extracellular enzyme kinetics, respectively. SOC is soil organic carbon. See the *Hypothesis testing* section within the Methods for a detailed explanation and rationale of the hypotheses tested.

Figure 2. Effects of mean annual temperature (MAT) and microbial carbon use efficiency (CUE) on mass-specific soil heterotrophic respiration rates (R_{mass}) assessed in a laboratory incubation of 110 dryland soils (a) and with soil organic carbon (SOC) models (b-e). (a) The effect sizes were estimated using coefficients from a linear mixed-effect model between R_{mass} , MAT, assay temperature and microbial biomass (a). SOC models simulated R_{mass} using positive vs. negative CUE–MAT relationships (corresponding to Hypothesis 1a and 1b in Figure 1, respectively) in first-order and microbial-explicit SOC models (b-e). R_{mass} was consistently assessed between the soil incubations and models with carbon substrate in excess of microbial demand, a short time scale (10 h), and three assay temperatures (10, 20 and 30°C). We provide the relative changes (%) in measured and simulated R_{mass} , as compared to equilibrium values at 20°C (the gray line), to facilitate comparisons between hypotheses and SOC models.

Figure 3. Longer-term (one year) simulated effects of mean annual temperature (MAT) on mass-specific soil heterotrophic respiration rates (R_{mass}), microbial biomass carbon (MBC) and soil organic carbon (SOC). The SOC models were simulated using a positive microbial carbon use efficiency (CUE)–MAT relationship. R_{mass} was consistently assessed in the soil incubations and models with carbon substrate in excess of microbial demand and three assay temperatures (10, 20 and 30°C). We provide the relative changes (%) in measured and simulated R_{mass} , as compared to equilibrium values at 20°C (the gray line), to facilitate comparisons between hypotheses and SOC models.

Figure 4. Effects of mean annual temperature (MAT) and micro-environment (vegetated patches vs. open areas) on mass-specific soil heterotrophic respiration rates (R_{mass}) assessed in a laboratory incubation of soils from 110 dryland sites (**a**) and soil organic carbon models (**b-e**). The effect sizes were estimated using the same approach described in Figure 2a (**a**). Carbon use efficiency (CUE) for vegetated patches = $\exp[-1.58 + 0.015 \times (\text{MAT} - 10)]$ (**b-c**). CUE for vegetated patches = $\exp(-1.58 + 0.015 \times \text{MAT}) \times 1.45$ (**d-e**). R_{mass} was consistently assessed between the soil incubations and models with carbon substrate in excess of microbial demand, a short time scale (10 h), and three assay temperatures (10, 20 and 30°C). To simplify the interpretation, only the results for 20°C are shown (they are representative of the general patterns observed). We provide the relative changes (%) in measured and simulated R_{mass} , as compared to equilibrium values at 20°C (the gray line), to facilitate comparisons between hypotheses and SOC models.

Figure 5. Effects of mean annual temperature (MAT) and soil microbial extracellular enzyme kinetics on mass-specific soil heterotrophic respiration rates (R_{mass}), simulated by the microbial-explicit soil organic carbon model under scenarios of carbon substrate in excess of microbial demand. Hypothesizing that both V_{max} (the maximal activity) and K_m (half-saturation constant) decrease with MAT, i.e. Hypothesis 2a in Figure 1b (a). Hypothesizing that both K_m and V_{max} increase with MAT, i.e. Hypothesis 2b in Figure 1b (b). The first-order soil organic carbon model does not explicitly include K_m and V_{max} , so no simulation is given. R_{mass} was consistently assessed between the soil incubations and models with carbon substrate in excess of microbial demand, a short time scale (10 h), and three assay temperatures (10, 20 and 30°C).

Figure 6. Comparison of simulated and measured mass-specific soil heterotrophic respiration rates (R_{mass}), assuming that a soil assay/incubation temperature matched the mean annual temperature (MAT) value of its source site. In our soil organic carbon (SOC) models, we incorporated two hypothetical CUE-MAT relationships i.e. positive and negative (Figure 1b). R_{mass} was assessed in models with carbon substrate in excess of microbial demand and a short time scale (10 h), consistent with the empirical approach of Dacal et al. (2019). We provide the relative changes (%) in measured and simulated R_{mass} , as compared to their reference values at 20°C, to facilitate comparisons between soil incubations and model simulations.

Supporting information

Additional Supporting Information, including Figures S1-S5, Method S1, Tables S1-S2, and R code for the soil organic carbon models can be found online in the supporting information tab for this article.







